

# Blood glucose and liver glycogen in the rat

## Effects of chronic ethanol consumption and its withdrawal on the diurnal rhythms

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In rats, chronic ethanol consumption results in moderate hypoglycemia with no great changes in the diurnal patterns of blood glucose and hepatic glucose. Marked alterations of the diurnal rhythm of liver glycogen were observed in ethanol-fed animals, the phase of the rhythm being shifted and the daily mean and minimum levels being lowered. After withdrawal of ethanol consumption, blood glucose remained at a nearly constant level for 18 h, but then decreased sharply causing the liver/blood gradient of glucose to increase up to 4. Liver glycogen varied with a small amplitude and a nearly inverted phase in withdrawn rats as compared to controls.

<i>Ethanol consumption</i>	<i>Ethanol withdrawal</i> <i>Glucose gradient</i>	<i>Circadian rhythm</i> <i>(Rat liver, Blood)</i>	<i>Glycogen</i>	<i>Glucose</i>
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### 1. INTRODUCTION

In [1] we showed that chronic ethanol consumption by rats resulted in moderate hypoglycemia. However, 24 and 48 h after withdrawal of ethanol consumption, the blood glucose concentration was not normalized; rather, it decreased drastically [1], whereas liver glycogen was at the normal or a higher level [1,2]. Thus, the development of severe hypoglycemia was accompanied by accumulation of liver glycogen during the 24 h period after ethanol withdrawal. These unexpected findings enabled us to study the diurnal dynamics of blood glucose and liver glycogen after chronic ethanol consumption and withdrawal in rats.

### 2. MATERIALS AND METHODS

1-month-old male rats of the Wistar strain were used weighing 100–110 g at the start of experimentation. They were allowed free access to a low-calorie diet (see [1]) and divided into 3 groups.

Group 1 ( $n = 36$ ) was provided with water ad libitum acting as control. The two other groups received 10% (w/w) ethanol as the sole drinking liquid. When all animals reached 4.5 months of age (in February, sunlight between 07.00 and 18.00 h), 4 control and 2 ethanol-fed rats were decapitated at 10.00–10.10 h and every 3 h over the 24 h period. 1 week later, 2 other ethanol-fed rats were killed every 3 h over the 24 h period, as well as 4 animals of group 3 ( $n = 36$ ) in which alcohol consumption was withdrawn throughout the 24 h experimental period, water being substituted for ethanol.

For biochemical analysis, two pairs of ethanol-fed rats killed at 1-week intervals were combined and designated group 2 ( $n = 32$ ).

All materials and methods used have been described in [1].

### 3. RESULTS

#### 3.1. Liver glycogen

In control rats (killed early in Spring, see section 2) liver glycogen shows a significant circadian rhythm with an acrophase at 01.00 h and a nadir at 13.00–6.00 h (fig.1A). The daily mean level of glycogen is 258  $\mu\text{mol}$  glycosyl residues/g liver wet mass. These findings are in agreement with those given in [3–5] and identical with our data on glycogen variation in rats killed later in Autumn [6].

Chronic ethanol consumption results in substantial changes in the diurnal dynamics of glycogen (fig.1B). The maximum appears to be shifted to 10.00 h and the minimum level is as low as 75  $\mu\text{mol}/\text{g}$ , remaining constant over the rest of the day and the daily mean level decreases to 136  $\mu\text{mol}/\text{g}$ .

Replacement of ethanol with water is accompanied by glycogen accumulation in the liver (fig.1C). The rhythmic amplitude (as a percentage of the daily mean level) is reduced from 58 to 25%. During the period 10.00–16.00 h, glycogen in-

creases to the maximum level of 330  $\mu\text{mol}/\text{g}$ , and by 01.00 h decreases to the minimum level of 210  $\mu\text{mol}/\text{g}$ , i.e. the diurnal rhythm of glycogen in withdrawn rats is nearly the opposite of that in controls (see fig.1A) over the period 10.00–04.00 h.

Throughout the 24 h period after withdrawal of ethanol consumption, liver glycogen is maintained at the high level.

#### 3.2. Blood and hepatic glucose concentrations

In control rats, blood and hepatic glucose concentrations, in terms of  $\mu\text{mol}$  per g blood or tissue, show similar diurnal variations (fig.2A); both have diurnal maxima at 10.00–13.00 h and minima during darkness. At any time of day glucose concentration in the blood is approximately that in the liver.

Chronic ethanol consumption causes only slight changes in the diurnal patterns of blood and hepatic glucose concentrations, therefore the ab-

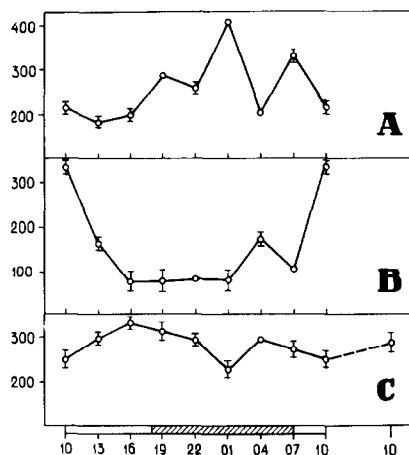


Fig.1. Effects of chronic ethanol consumption and withdrawal on the diurnal rhythm of liver glycogen in rats. (A) Control, (B) after 3.5 months ethanol consumption, (C) during the 1st day after withdrawal of ethanol consumption. Values are given as means  $\pm$  SD for 4 animals at each time point and are expressed in  $\mu\text{mol}$  glycosyl residues per g liver wet mass. The time of day is indicated on the abscissa, the dark period being shaded. The number 10 on the right represents 10.00 h of the 3rd day (48 h after ethanol withdrawal).

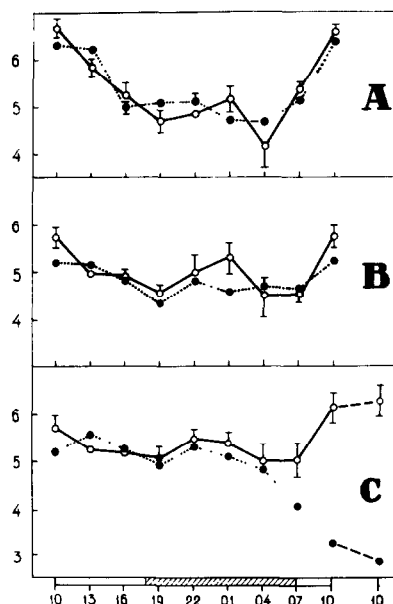


Fig.2. Effects of chronic ethanol consumption and withdrawal on the diurnal rhythms of blood and hepatic glucose levels in rats. Values are given as means  $\pm$  SD for 4 animals in each time point (except for 3 animals at 04.00 and 07.00 h in C, blood glucose) and are expressed in  $\mu\text{mol}/\text{g}$  wet mass of blood or liver. Hepatic (—) and blood (···) glucose levels. For other details, see legend to fig.1.

solute glucose concentration in the blood remains nearly the same as that in the liver throughout the day (fig.2B).

Both blood and hepatic glucose concentrations are maintained at almost constant levels during the 18 h period after substitution of water for ethanol (i.e. between 10.00 and 04.00 h) and in absolute terms are similar at any time within this period (fig.2C). Subsequently, the blood glucose concentration dropped drastically, reaching a minimum level of  $3 \mu\text{mol/g}$  blood at 10.00 h of the second day, and being maintained at this low level for the next 24 h. In contrast, hepatic glucose concentration increases by the end of the first day and remains at such a high level to the end of the second day after ethanol withdrawal (fig.2C).

### 3.3. Liver/blood gradient of glucose

We calculated glucose concentrations in blood plasma water and in liver cytosolic water using the formulas given in [1]. The results are depicted in fig.3. The ratio of glucose concentration in liver cytosolic water to that in plasma water varies within a narrow range, 1.15–1.62 for controls (fig.3A) and 1.30–1.72 for ethanol-fed rats (fig.3B). These are in reasonable agreement with the value of 1.0 expected for an equilibrium distribution of glucose between liver cytosol and

blood plasma [7–9]. The same is true for animals in group 3: the liver water/plasma water gradient of glucose is 1.3–1.6 over the 18 h period after ethanol withdrawal (fig.3C). However, by the end of the 24 h period after withdrawal, the ratio increases sharply to a value of 3.5 and by the end of the 48 h period, to 4.2 (fig.3C).

The wet mass (in g) of rat liver from all 3 groups differs insignificantly, being  $8.6 \pm 0.6$  (range, 7.6–10.1) for controls,  $7.6 \pm 0.4$  (6.0–9.2) for ethanol-fed and  $8.5 \pm 1.0$  (7.0–10.6) for withdrawn rats. Dry liver mass accounts for  $70.3 \pm 2.4$ ,  $69.6 \pm 3.8$  and  $71.7 \pm 5.3\%$ , respectively, and changes insignificantly over the day. These results are consistent with the findings of Lee and Hosein [10] for chronic alcohol-fed rats and show that the changes observed in hepatic glucose level are not due to alterations in hepatocyte cytosolic water. It is also obvious that the decrease in blood glucose concentration in withdrawn rats by a factor of 2 cannot be explained by a decrease in the relative amount of plasma water.

## 4. DISCUSSION

Our results support and expand current knowledge of alcohol effects on carbohydrate energy metabolism ([1] and references therein). Hypoglycemia was shown previously to develop in animals and human subjects upon ethanol consumption. It is clear from the present work that alcoholic hypoglycemia in rats is observed only between 10.00 and 13.00 h (fig.2A,B), while the differences between control and ethanol-fed animals in blood glucose level is usually insignificant at other times of the day.

In chronic ethanol consumption, the diurnal rhythm of liver glycogen is also changed. The maximum of liver glycogen achieved at 01.00 h in control animals is shifted to 10.00 h after chronic ethanol consumption. At other times of the day the liver glycogen concentration in ethanol-fed animals does not exceed  $160\text{--}170 \mu\text{mol/g}$ ; the minimum level is as low as  $75 \mu\text{mol/g}$  and is maintained during the period 16.00–01.00 h, when liver glycogen is accumulated in control rats.

An important point to note in the effects of ethanol consumption is the marked quantitative difference between control and ethanol-fed rats in liver glycogen level at different times of the day. In

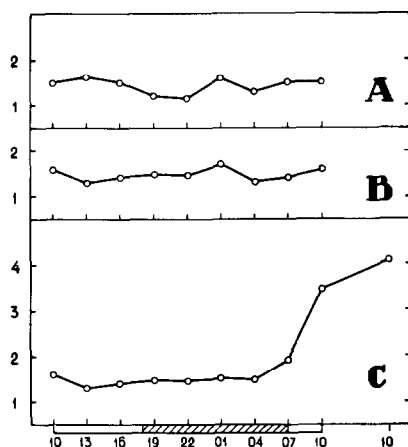


Fig.3. Effects of chronic ethanol consumption and withdrawal on the diurnal rhythm of the liver cytosolic water/blood plasma water gradient of glucose in rats. Values are given as means for 4 animals at each time point (except for 3 animals at 04.00 and 07.00 h in C).

Other details as in fig.1.

fact, liver glycogen in ethanol-fed rats at 07.00 and 16.00–01.00 h is 3–5-fold lower, and at 10.00 h is 1.5-fold higher than that in control animals, while at 13.00 and 04.00 h liver glycogen is the same in control and ethanol-fed animals. These data are suitable for explaining some contradictory observations given in the literature. Thus, Gordon [2] reported that liver glycogen was reduced 3-fold upon ethanol consumption by rats, while Antal et al. [11] observed an increase in liver glycogen after acute ethanol administration, and according to others [12] no changes in liver glycogen were observed in rats injected with acetaldehyde chronically. These authors did not state the time of day at which their animals were killed. The temporal aspect of the ethanol effect on liver glycogen ought to be accounted for in the interpretation of experimental results.

Glucose has long been known to distribute freely and uniformly between the liver and blood [7–9]. Our data in figs 2A and 3A are in agreement with this view, the uniform distribution of glucose between the liver cytosol and blood plasma being maintained over the 24 h period. Chronic ethanol consumption does not affect this pattern of glucose distribution (figs 2B,3B).

More drastic disturbances in carbohydrate homeostasis take place after ethanol withdrawal than upon ethanol consumption. The diurnal rhythm of liver glycogen becomes almost opposite in phase as compared with that in controls, and the rhythmic amplitude is sharply reduced. Glucose concentrations remain the same in both blood and liver and upon ethanol consumption and during 18 h after ethanol withdrawal. Later, hypoglycemia is emphasized inasmuch as large glycogen stores accumulate in the liver and hepatic glucose

concentration is increased somewhat. In conclusion, this and previous work show for the first time that the liver/blood barrier for glucose arises during the 24 h period after withdrawal of ethanol consumption. The damage in free glucose penetration from the liver into the circulation results in severe hypoglycemia despite the large stores of liver glycogen.

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